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RESEARCH PAPER

Nonequilibrium Statistical Model of Active Transport of Ions and ATP Production in Mitochondria

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Abstract A model of the active transport of ions through internal membranes of mitochondria is proposed. If concentrations of ions in a cell are known, this model allows calculating concentrations of all main ions (H^+, Ca^{+2}, K^+, Mg^{2+}, Na^+, Cl^-) in the mitochondrion matrix and the resting potential across the membrane. The theoretical values satisfactorily agree with available experimental data on the concentrations and the potentials, including different operating regimes of the adenosine triphosphate (ATP) synthetase (the main regime, short circuiting or ATP synthetase blocking). The active transport of Mg^{2+} ions in exchange for protons was assumed. In accordance with the model, the ATP synthetase operation is possible only if the stoichiometric coefficient of protons is 3.

Keywords Active transport of ions · Mitochondria · ATP production · Resting potential · Mathematical model

1 Introduction

A nonequilibrium statistical model of the active transport of ions in biomembranes was described in [1–3]. This model satisfies the main requirements of nonequilibrium statistical physics [2] and allows predicting the resting potential at the membrane and concentrations of ions inside a cell from their assigned concentrations outside the cell.

In this study, the aforementioned approach was used to describe functioning of the internal membrane of mitochondria, on which adenosine triphosphate (ATP) is synthesized.

Although a number of papers are dedicated to problems of the structure, the active and passive transports of ions through the membrane, and synthesis of ATP in mitochondria [4–7], a variety of questions are still to be answered:

1. How many protons take part in a single event of ATP synthesis (two or three)?
2. Is the transport of protons during ATP synthesis relatively independent of the Krebs cycle or rigidly connected with this cycle, representing and represents a link in the chain of the Krebs cycle?

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3. Are there Mg^{+2} pumps on the internal membrane of mitochondria?
4. What is the role of various ions in the establishment of a sufficiently large electrical potential at the mitochondrion membrane?

Experimental data are available [9] concerning three basic ATPase regimes of mitochondria namely, (1) nominal, (2) short circuit (the proton channel is open), and (3) open circuit (the proton channel is blocked and ATP is not produced). However, mechanisms responsible for changes in measured values of the potential and the proton concentration remain unclear. These and other problems are rather difficult and expensive to solve in experiments, while theoretical studies deal with semiempirical formulas for the flow of ions through the mitochondrion membrane [4–8]. These formulas have some drawbacks. Firstly, they do not hold near the equilibrium, excluding some operating regimes of mitochondria from consideration. Secondly, these formulas involve a large number of unknowns, which make it difficult to understand processes of the transport in mitochondria in qualitative terms. One more drawback is the disregard of the electric neutrality of the environment. Therefore, the resting potential and the concentrations of ions in a mitochondrion cannot be determined independently.

In what follows, we shall discuss whether this model can be used to describe functioning of the internal membrane of mitochondria and, also, analyze its characteristics in terms of the problems stated above.

1.1 Systems of Active Transport of Ions in Mitochondria

The model of mitochondria at hand will take into account systems of active transport known from experiments (see, for example, [10]; Fig. 1):

1. The transport of protons by reactions of the Krebs cycle
2. The $3Na^{+}$–$3H^{+}$ exchanger that pumps sodium out of the mitochondrion
3. The K^{+}–H^{+} exchanger that pumps potassium out of the mitochondrion
4. The ATP synthetase of the F_1F_0 type that synthesizes ATP thanks to different electrochemical potentials of protons and, if ADP is deficient, hydrolyzes ATP and, thus, removes protons from the mitochondrion
5. The electrogenically reactive $3Na^{+}$–Ca^{+2} exchanger (sodium to the inside and calcium to the outside)
6. The Ca^{+}–$2H^{+}$ exchanger that pumps calcium out of the mitochondrion
7. The P^{-}–H^{+} exchanger that pumps phosphate ions into the mitochondrion
8. The electrogenically reactive ATP^{4-}–ADP^{3-} exchanger that pumps ADP^{3-} into the mitochondrion and ATP^{4-} outside the mitochondrion, into the cell cytoplasm

Channels are also available for some ions having variable permeability. We shall assume them to be closed. Transport of chlorine ions will be assumed to be passive only.

Some ions (H^{+}, Ca^{2+}, Na^{2+}) participate in several types of active transport systems. In this case, the ratio between the operation frequencies of these systems needs to be known to solve the problem. However, it is not known so far. The frequency ratios will serve as fitting parameters during the simulation process. To reduce the number of these parameters, we shall use the logic by which the structure of mitochondria (and all other living organisms) was formed during biological evolution so as to provide a maximum efficient and reliable system producing the main “fuel” (ATP) for living organisms and quickly responding to their varying requirements for ATP.

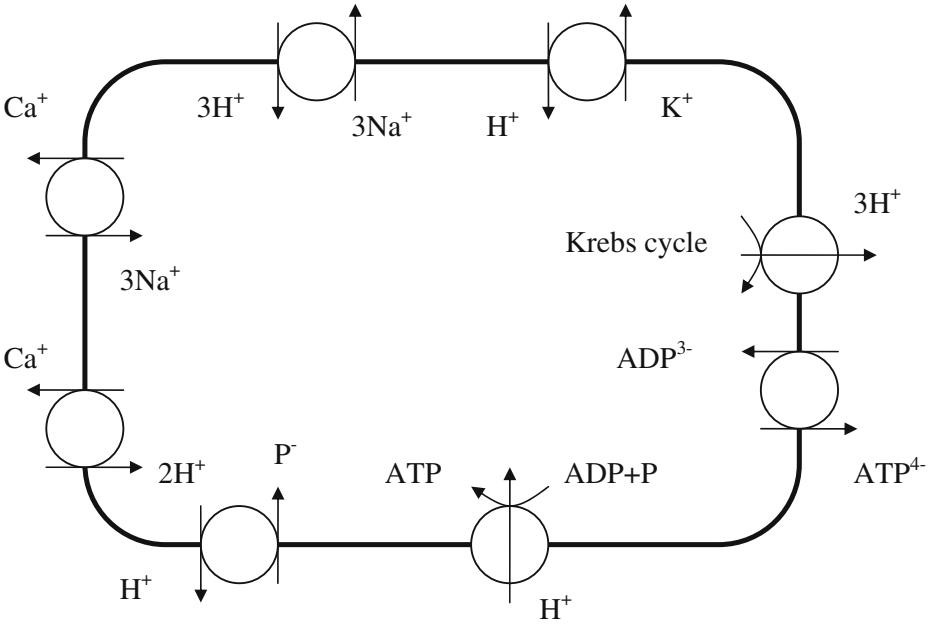

Fig. 1 Systems of active transport of ions in mitochondria

As with any control system [11, 12], ATP production system can be managed reliably if superfluous secondary effects are eliminated wherever possible (robust control) and the required minimum number of factors of adaptive control are preserved. Following this logic, it may be assumed that the electrochemical potential of protons is independent of all mechanisms of their passive transport (including the exchangers) through the internal membrane of mitochondria (robust control) when the unidirectional frequency of their transport in a Krebs cycle $\nu^{\mathrm{K}}_{\mathrm{i}\to\mathrm{o}}$ (the resulting frequency $\nu^{\mathrm{K}} = \nu^{\mathrm{K}}_{\mathrm{i}\to\mathrm{o}} - \nu^{\mathrm{K}}_{\mathrm{o}\to\mathrm{i}}$) is much smaller than any unidirectional frequency of their passive transport $\nu^{\mathrm{P}}_{\mathrm{i}\to\mathrm{o}}$ or $\nu^{\mathrm{P}}_{\mathrm{o}\to\mathrm{i}}$ (here, the subscripts i and o mean the inside and the outside of the mitochondrion, respectively). It may be assumed in this case that the difference of the electrochemical potentials of protons at the membrane, $\Delta\mu^{\mathrm{e}}_{\mathrm{H}}$, will be determined mainly by properties of protein molecules in the Krebs cycle and will depend little on the presence of the other channels of their transport, including the ATP synthetase. This thesis is supported by experimental data [9], which demonstrate that $\Delta\mu^{\mathrm{e}}_{\mathrm{H}}$ changes from 216 to 229 mV, i.e., by not more than 6%, when the ATP synthetase is blocked.

1.2 Model of Proton, Na^+, K^+, Ca^{2+}, and Mg^{2+} Transport. Resting Potential Calculation

Considering the logic described above and the active transport equations given in [1–3], let us write equations for each type of ion transported through the mitochondrion membrane. We shall dwell first on the transport of protons. In accordance with the concept of robust control, only one mechanism – the transport by the chemical reaction of the Krebs cycle – is chosen out of all mechanisms of the transport of protons for determination of the difference $\Delta\mu^{\mathrm{e}}_{\mathrm{H}}$. The passive transport and the involvement of H^+ ions in the exchangers

and the ATP synthetase are neglected. Then, in line with [1], the following equation can be written for the flow of protons in the Krebs cycle:

$$J_{\mathrm{H}} = C_1 \frac{\exp(\Delta\mu_{\mathrm{KR}} + 3\varphi)\left(n_{\mathrm{H}}^{\mathrm{i}}\right)^3 - \left(n_{\mathrm{H}}^{\mathrm{o}}\right)^3}{1 + \exp(\Delta\mu_{\mathrm{KR}} + 3\varphi - Q)}, \tag{1}$$

where $\Delta\mu_{\mathrm{KR}}$ is the dimensionless (in kT units) difference of the chemical potentials in the Krebs cycle, φ is the dimensional (in kT units) potential at the membrane, Q is the dimensional reaction heat in the Krebs cycle, $n_{\mathrm{H}}^{\mathrm{i}}$ and $n_{\mathrm{H}}^{\mathrm{o}}$ denote the proton concentrations on the inside and the outside of the mitochondrion, and C_1 is a constant proportional to the number of reaction systems in the Krebs cycle and the operation frequency $\nu_{\mathrm{i}\to\mathrm{o}}$. In the stationary state $J_{\mathrm{H}}=0$ and, therefore, from Eq. 1, it is possible to determine the ratio between the proton concentrations:

$$\frac{n_{\mathrm{H}}^{\mathrm{o}}}{n_{\mathrm{H}}^{\mathrm{i}}} = \exp\left(\frac{\Delta\mu_{\mathrm{KR}}}{3} + \varphi\right) \tag{2}$$

or, taking into account that $\Delta pH = \ln\frac{n_{\mathrm{H}}^{\mathrm{o}}}{n_{\mathrm{H}}^{\mathrm{i}}}$ and $\Delta\mu_{\mathrm{H}}^{\mathrm{e}} = \varphi + \Delta\mathrm{pH}$, one can write

$$\Delta\mu_{\mathrm{KR}} = -3\Delta\mu_{\mathrm{H}}^{\mathrm{e}}. \tag{3}$$

If the passive transport is taken into account, the emf (electromotive force) $\Delta\mu_{\mathrm{H}}^{\mathrm{e}}$ of the ATP synthesis decreases.

We shall use the same concept of robust control in describing the transport of other ions. One may think that the passive transport of Na^+ and Mg^+ ions, whose concentrations in the cell are relatively small, should have little influence on $\Delta\mu_{\mathrm{H}}^{\mathrm{e}}$ because of a sufficiently large number of $3Na^+$–$3H^+$ and Mg^+–$2H^+$ exchangers. In this case, the passive transport becomes less significant because the unidirectional transport frequencies in the exchangers, $\nu_{\mathrm{i}\to\mathrm{o}}^{\mathrm{Na}}$ and $\nu_{\mathrm{i}\to\mathrm{o}}^{\mathrm{Mg}}$, are much higher than the corresponding frequencies of the passive transport.

For the adaptive control of the membrane potential, it is necessary to ensure competitiveness of currents of the active and passive transport of positive ions having the maximum concentration (K^+ ions). This statement is supported by experimental data [10, 13], which suggest that the passive transport of potassium ions is significant for maintaining their internal concentration. Ca^{+2} ions regulating the ATP synthetase frequency play a special role [14–18]. Therefore, they should be carried by the passive transport through the internal membrane of the mitochondrion.

The reliable performance of mitochondria also depends on a small osmotic pressure differential across the membrane.

Considering this requirement, the total concentrations of all ions on both sides of the membrane should be maintained nearly equal. The main ions with a maximum concentration are nonpenetrating negative A^- ions and K^+ cations. Therefore, the requirement

$$n_{\mathrm{A}}^{\mathrm{i}} + n_{\mathrm{K}}^{\mathrm{i}} \approx n_{\mathrm{A}}^{\mathrm{o}} + n_{\mathrm{K}}^{\mathrm{o}} \tag{4}$$

should be fulfilled on the one hand, and, considering the neutrality condition (neglecting the other ions), the relationship

$$n_A^i \approx n_K^i \ \text{и} \ n_A^o \approx n_K^o \tag{5}$$

should be met on the other hand.

If the operating regime of the H^+–K^+ exchanger is far from the saturation of the two sorption centers, the ratio between the potassium ion concentrations will change, depending on the capacity of the ATP synthetase and, correspondingly, the difference ΔpH across the membrane, by the law (if the passive transport of K^+ can be neglected)

$$\frac{n_{\mathrm{K}}^{\mathrm{i}}}{n_{\mathrm{K}}^{\mathrm{o}}} = \frac{n_{\mathrm{H}}^{\mathrm{o}}}{n_{\mathrm{H}}^{\mathrm{i}}}, \tag{6}$$

leading to an extremely large ratio between the potassium concentrations across the membrane and a too large variation of this ratio if $n_{\mathrm{H}}^{\mathrm{i}}/n_{\mathrm{H}}^{\mathrm{o}}$ changes. The operation of the ATP production machine and its control are satisfied more by the operating regime of the H^+–K^+ exchanger when a high concentration of K^+ ions ensures saturation of the K^+ sorption centers on both sides of the membrane and the exchanger flow is proportional to the concentration difference $n_{\mathrm{H}}^{\mathrm{o}} - n_{\mathrm{H}}^{\mathrm{i}}$.

Considering these general observations, let us write equations for the ion transport through the membrane. We shall take first the transport of potassium ions:

$$J_{\mathrm{K}} = -C_2\left(n_{\mathrm{H}}^{\mathrm{o}} - n_{\mathrm{H}}^{\mathrm{i}}\right) + P_{\mathrm{K}}\left(n_{\mathrm{K}}^{\mathrm{o}} - n_{\mathrm{K}}^{\mathrm{i}}\exp(\varphi)\right), \tag{7}$$

where C_2 is a constant proportional to the number of H^+–K^+ exchangers and the unidirectional operation frequency $v_{\mathrm{i}\to\mathrm{o}}^{\mathrm{K}}$, P_{K} is the membrane permeability by potassium ions, and J_{K} is the resulting flow of potassium ions.

Because the concentration of potassium ions in mitochondria is much larger than the concentration of other positive ions, the neutrality condition in mitochondria is reduced, as the first approximation, to the equality

$$n_{\mathrm{A}}^{\mathrm{i}} = n_{\mathrm{K}}^{\mathrm{i}}, \tag{8}$$

where $n_{\mathrm{A}}^{\mathrm{i}}$ is the concentration of nonpenetrating negative ions in mitochondria.

Substituting Eq. 8 into Eq. 7 and considering that in the stationary state $J_{\mathrm{K}}=0$, it is easy to deduce the equation for the mitochondrion membrane potential:

$$\varphi = \ln\left[\frac{P_{\mathrm{K}}n_{\mathrm{K}}^{\mathrm{o}} + C_2 n_{\mathrm{H}}^{\mathrm{i}}}{P_{\mathrm{K}}n_{\mathrm{A}}^{\mathrm{i}} + C_2 n_{\mathrm{H}}^{\mathrm{i}}\exp\left(\frac{\Delta\mu_{\mathrm{KR}}}{3}\right)}\right]. \tag{9}$$

If the membrane permeability by potassium ions is so small that $P_{\mathrm{K}}n_{\mathrm{K}}^{\mathrm{o}} << C_2 n_{\mathrm{H}}^{\mathrm{i}}$ and $P_{\mathrm{K}}n_{\mathrm{A}}^{\mathrm{i}} << C_2 n_{\mathrm{H}}^{\mathrm{i}}\exp(\frac{\Delta\mu_{\mathrm{KR}}}{3})$, we obtain the upper estimate of the potential:

$$\varphi_{\mathrm{m}} = -\frac{\Delta\mu_{\mathrm{KR}}}{3} = \Delta\mu_{\mathrm{H}}^{\mathrm{e}}. \tag{10}$$

If passive permeability of potassium is taken into account, the potential decreases relative to its maximum value, and ΔpH is formed.

The experimental data [9] demonstrate that in the nominal operating regime ΔpH=−1.92 and φ=−6.7; that is, in accordance with Eq. 10, passive transport is relatively insignificant.

Let us turn to the transport of calcium ions. Because these ions are responsible for regulation, all the components, including passive transport, are retained in the expression for the flow:

$$J_{\mathrm{Ca}} = -C_3\left[n^{\mathrm{i}}_{\mathrm{Ca}}\left(n^{\mathrm{o}}_{\mathrm{H}}\right)^2 - n^{\mathrm{o}}_{\mathrm{Ca}}\left(n^{\mathrm{i}}_{\mathrm{H}}\right)^2\right] - C_4\left[n^{\mathrm{i}}_{\mathrm{Ca}}\left(n^{\mathrm{o}}_{\mathrm{Na}}\right)^2 - n^{\mathrm{o}}_{\mathrm{Ca}}\left(n^{\mathrm{i}}_{\mathrm{Na}}\right)^2\right] + P_{\mathrm{Ca}}\left(n^{\mathrm{o}}_{\mathrm{Ca}} - n^{\mathrm{i}}_{\mathrm{Ca}}\exp(2\varphi)\right), \tag{11}$$

where C_3 is a constant proportional to the number of $2H^+$–Ca^{2+} exchangers and the unidirectional frequency of their operation, C_4 is a constant proportional to the number of $3Na^+$–Ca^{2+} exchangers and the unidirectional frequency of their operation, and P_{Ca} is the membrane permeability of Ca^{+2} ions.

If C_3, C_4, and P_{Ca} are unknown, the concentration ratio of Ca^{+2} ions cannot be obtained on the condition $J_{\mathrm{Ca}}=0$. It is clear, however, that if $P_{\mathrm{Ca}}>>(C_3, C_4)$, $n^{\mathrm{i}}_{\mathrm{Ca}}$ will be $\exp(2\times 6.7)$ times larger than $n^{\mathrm{o}}_{\mathrm{Ca}}$, leading to the rupture of the cell due to osmotic pressure. If $C_3>>(P_{\mathrm{Ca}}, C_4)$, the ratio of the calcium concentrations at $\Delta\mathrm{pH}=-1.92$ will be

$$\frac{n^{\mathrm{o}}_{\mathrm{Ca}}}{n^{\mathrm{i}}_{\mathrm{Ca}}} = \exp(2\times 1.92) = 50.2, \tag{12}$$

whereas, from the experiment, we have $\frac{n^{\mathrm{o}}_{\mathrm{Ca}}}{n^{\mathrm{i}}_{\mathrm{Ca}}} \approx 1$. This fact suggests, in line with Eq. 11, that the passive transport of calcium cannot be neglected. This observation confirms experimental data concerning the role of passive transport of Ca^{+2} [4, 10, 14–18].

Sodium is carried by two active systems operating in opposite directions under physiological conditions. We shall take the outward flow of sodium as its main flow and the inward flow of sodium as its regulating (secondary) flow. This assumption is due to robust control because it is required that sodium does not influence the establishment of the membrane potential. Therefore, the following expression can be written for sodium under stationary conditions:

$$J_{\mathrm{Na}} = C_5\left[\left(n^{\mathrm{i}}_{\mathrm{Na}}\right)^3\left(n^{\mathrm{o}}_{\mathrm{H}}\right)^3 - \left(n^{\mathrm{o}}_{\mathrm{Na}}\right)^3\left(n^{\mathrm{i}}_{\mathrm{H}}\right)^3\right] = 0. \tag{13}$$

Then, the concentration ratio of sodium ions is

$$\frac{n^{\mathrm{o}}_{\mathrm{Na}}}{n^{\mathrm{i}}_{\mathrm{Na}}} = \exp(-\Delta\mathrm{pH}) = 7.3. \tag{14}$$

Chlorine is carried by passive transport, and therefore, we can write

$$n^{\mathrm{i}}_{\mathrm{Cl}} = n^{\mathrm{o}}_{\mathrm{Cl}}\exp(\varphi). \tag{15}$$

As to the transport of Mg^{+2} ions, experimental studies (see the review [13]) have not revealed so far the active transport of Mg^{+2} in mitochondria. Still, such a system should be available. The necessity of this system was discussed in [13] and was substantiated by the need to provide a low osmotic pressure differential across the membrane. Moreover, the concentration of Mg^{+2} ions should not be over $0.1n^{\mathrm{i}}_{\mathrm{K}} = 10$ mM to exclude a considerable effect on the electric potential in accordance with Eq. 4.

For the magnesium concentration to be low, these ions should be carried through the mitochondrion membrane by active transport. If we assume that this system is presented by

an exchanger analogous to the calcium exchanger, the following equation can be written for magnesium ions:

$$J_{\mathrm{Mg}} = C_6\left[n^{\mathrm{i}}_{\mathrm{Mg}}\left(n^{\mathrm{o}}_{\mathrm{H}}\right)^2 - n^{\mathrm{o}}_{\mathrm{Mg}}\left(n^{\mathrm{i}}_{\mathrm{H}}\right)^2\right] = 0. \tag{16}$$

In accordance with this equation,

$$\frac{n^{\mathrm{o}}_{\mathrm{Mg}}}{n^{\mathrm{i}}_{\mathrm{Mg}}} = \exp(2 \times 1.92) = 50.2. \tag{17}$$

The experimental values [19, 20] are $n^{\mathrm{o}}_{\mathrm{Mg}} \approx 1$ mM and $n^{\mathrm{i}}_{\mathrm{Mg}} \approx 0.1$ mM. This is in agreement with the theoretical concentration ratio Eq. 17.

1.3 Model of ATP Production

Let us consider the exchanger carrying ATP^{-4} ions to cells from mitochondria and ADP^{-3} ions to mitochondria, which was revealed in experiments [10]. By this exchanger, ATP synthesized in mitochondria is carried to cells, while the initial reagent of the ADP synthesis passes to the matrix. The ATP flow to cells can be written as in [1–3]

$$J_{\mathrm{ATP}} = C_7\left[\frac{n^{\mathrm{i}}_{\mathrm{ATP}} n^{\mathrm{o}}_{\mathrm{ADP}} \exp(\varphi) - n^{\mathrm{o}}_{\mathrm{ATP}} n^{\mathrm{i}}_{\mathrm{ADP}}}{1 + \exp(\varphi)}\right]. \tag{18}$$

Unlike other ions, ATP^{-4} (ADP^{-3}) have a source in mitochondria (cells) and a sink in cells (mitochondria). Therefore, the flow J_{ATP} is not zero in the stationary state. Still, it is reasonable to consider the variant when the source and the sink of ATP are nearly zero (for example, the organism is at rest). The concentrations at rest will be marked with an asterisk. Then, we have from the condition J_{ATP}=0:

$$\frac{n^{\mathrm{i}*}_{\mathrm{ATP}}}{n^{\mathrm{o}*}_{\mathrm{ATP}}}\frac{n^{\mathrm{o}*}_{\mathrm{ADP}}}{n^{\mathrm{i}*}_{\mathrm{ADP}}} = \exp(-\varphi). \tag{19}$$

P^- ions are also required for ATP synthesis. It is known from experiments [10] that the mitochondrion membrane has the H^+–P^- exchanger for organization of P^- flow to the mitochondrion. Let us write the equation for the flow through this exchanger:

$$J_{\mathrm{P}} = C_8\left[n^{\mathrm{o}}_{\mathrm{H}} n^{\mathrm{o}}_{\mathrm{P}} - n^{\mathrm{i}}_{\mathrm{H}} n^{\mathrm{i}}_{\mathrm{P}}\right]. \tag{20}$$

If the flow is nearly zero, we obtain

$$\frac{n^{\mathrm{o}*}_{\mathrm{P}}}{n^{\mathrm{i}*}_{\mathrm{P}}} = \frac{n^{\mathrm{i}}_{\mathrm{H}}}{n^{\mathrm{o}}_{\mathrm{H}}} = \exp(\Delta\mathrm{pH}). \tag{21}$$

In accordance with the definition of the difference of chemical potentials of the ATP↔ADP+P reaction on the inside and the outside of mitochondria, we can write

$$\frac{n^{\mathrm{i}*}_{\mathrm{ATP}}}{n^{\mathrm{i}*}_{\mathrm{ADP}} n^{\mathrm{i}*}_{\mathrm{P}}}\frac{n^{\mathrm{o}*}_{\mathrm{ADP}} n^{\mathrm{o}*}_{\mathrm{P}}}{n^{\mathrm{o}*}_{\mathrm{ATP}}} = \exp\left(\Delta\mu^{\mathrm{i}*}_{\mathrm{A}} - \Delta\mu^{\mathrm{o}*}_{\mathrm{A}}\right). \tag{22}$$

Substituting Eqs. 19 and 21 into Eq. 22 gives

$$\Delta\mathrm{pH} - \varphi = \Delta\mu^{\mathrm{i}*}_{\mathrm{A}} - \Delta\mu^{\mathrm{o}*}_{\mathrm{A}}. \tag{23}$$

Considering that ΔpH=−1.92 and φ=−6.7, we have

$$\Delta\mu_{\mathrm{A}}^{\mathrm{i}*} - \Delta\mu_{\mathrm{A}}^{\mathrm{o}*} = 4.78. \tag{24}$$

Values of these quantities were measured in experiments [9]: $\Delta\mu_{\mathrm{A}}^{\mathrm{i}} = 25.6$ and $\Delta\mu_{\mathrm{A}}^{\mathrm{o}} = 20.0$. The comparison shows that the values of $\Delta\mu_{\mathrm{A}}^{\mathrm{i}} - \Delta\mu_{\mathrm{A}}^{\mathrm{o}}$ and $\Delta\mu_{\mathrm{A}}^{\mathrm{i}*} - \Delta\mu_{\mathrm{A}}^{\mathrm{o}*}$ differ little. This observation, on the one hand, confirms the theoretical calculations and, on the other hand, suggests that the distribution of potentials under physiological conditions approaches their distribution at rest.

Let us simulate functioning of the ATP synthetase. Considering the concept of robust control, we shall assume that the consumption of H^+ ions in the ATP synthetase does not influence the electrochemical potential of protons $\Delta\mu_{\mathrm{H}}^{\mathrm{e}} = \varphi + \Delta\mathrm{pH}$. In line with [21], the expression for the flow of protons to cells can be written in the form

$$J_{\mathrm{H}} = C_9 \frac{\exp\left(\Delta\mu_{\mathrm{A}}^{\mathrm{i}} + b\varphi\right)\left(n_{\mathrm{H}}^{\mathrm{i}}\right)^b - \left(n_{\mathrm{H}}^{\mathrm{o}}\right)^b}{1 + \exp\left(\Delta\mu_{A}^{i} + b\varphi - Q\right)}, \tag{25}$$

where C_9 is a constant proportional to the number of ATP synthetases in a mitochondrion and the unidirectional frequency of their operation, Q is the ATP hydrolysis reaction heat, and b is the stoichiometric coefficient.

Taking into account that

$$\exp\left(\Delta\mu_{\mathrm{A}}^{\mathrm{i}} + b\varphi - Q\right) << 1 \tag{26}$$

and $\ln\left(\frac{n_{\mathrm{H}}^{\mathrm{i}}}{n_{\mathrm{H}}^{\mathrm{o}}}\right) = \Delta\mathrm{pH}$ in the conditions at hand, we can write instead of Eq. 25:

$$J_{\mathrm{H}} = C_9\left[\exp\left(\Delta\mu_{\mathrm{A}}^{\mathrm{i}} + b\varphi + b\Delta\mathrm{pH}\right) - 1\right] = C_9\left[\exp\left(\Delta\mu_{\mathrm{A}}^{\mathrm{i}} + b\Delta\mu_{\mathrm{H}}^{\mathrm{e}}\right) - 1\right]. \tag{27}$$

In accordance with Eq. 27, the proton flow is directed to the cell if $\Delta\mu_{\mathrm{A}}^{\mathrm{i}} > \left|b\Delta\mu_{\mathrm{H}}^{\mathrm{e}}\right|$; in this case, ATP is hydrolyzed. Oppositely, if $\Delta\mu_{\mathrm{A}}^{\mathrm{i}} < \left|b\Delta\mu_{\mathrm{H}}^{\mathrm{e}}\right|$, ATP is synthesized, and the proton flow is directed to the mitochondrion. The stoichiometric coefficient b is determined from the ATP synthesis condition:

$$b > \frac{\Delta\mu_{\mathrm{A}}^{\mathrm{i}}}{\left|\Delta\mu_{\mathrm{H}}^{\mathrm{e}}\right|} = \frac{25.6}{8.6} = 2.98. \tag{28}$$

Obviously, the coefficient b cannot be equal to 2. That is, taking into account the requirement of the high efficiency of biological machines, three protons should be involved in the ATP synthesis reaction. Classical experiments concerned with measurements of the potential and ΔpH give the following values for three operating regimes of mitochondria:

1. The normal physiological condition (ATP is produced):

$$\varphi_1 = -6.7,\ (\Delta\mathrm{pH})_1 = -1.92,\ \left(\Delta\mu_{\mathrm{H}}^{\mathrm{e}}\right)_1 = -8.62. \tag{29}$$

2. Short circuit (the proton channel is open):

$$\varphi_1 = -3.16,\ (\Delta\mathrm{pH})_2 = 1,\ \left(\Delta\mu_{\mathrm{H}}^{\mathrm{e}}\right)_2 = -2.16. \tag{30}$$

3. The circuit is open (the proton channel is blocked; ATP is not produced):

$$\varphi_1 = -5.4,\ (\Delta\mathrm{pH})_3 = -3.8,\ \left(\Delta\mu_{\mathrm{H}}^{\mathrm{e}}\right)_3 = -9.2. \tag{31}$$

Let us see how the measured values change in terms of the proposed theoretical model. The small difference of $\left(\Delta\mu_{\mathrm{H}}^{\mathrm{e}}\right)_1$ and $(\Delta\mu_{\mathrm{H}}^{\mathrm{e}})_3$ confirms the assumption of our model that the proton flow in the ATP synthetase should not have a considerable effect on the emf value. Indeed, $\frac{(\Delta\mu_{\mathrm{H}}^{\mathrm{e}})_3-(\Delta\mu_{\mathrm{H}}^{\mathrm{e}})_1}{(\Delta\mu_{\mathrm{H}}^{\mathrm{e}})_3}=6.3\%$. If the circuit is open and the loss of protons in the exchangers is neglected, the difference of the chemical potentials of the Krebs cycle may be taken equal to $\Delta\mu_{\mathrm{KR}}=-3\left(\Delta\mu_{\mathrm{H}}^{\mathrm{e}}\right)_3=27.6$ in accordance with Eq. 3. If the ATP synthetase is engaged, the flow (Eq. 1) is provided by the difference $\Delta\mu_{\mathrm{KR}}-3\left|\left(\Delta\mu_{\mathrm{H}}^{\mathrm{e}}\right)_1\right|=27.6-3\times 8.62=1.8$. To verify this statement, rearrange Eq. 1 to the form

$$J_{\mathrm{H}}=C_1[\exp(\Delta\mu_{\mathrm{KR}}+3\varphi+3\Delta\mathrm{pH})-1]=C_1\left[\exp\left(\Delta\mu_{\mathrm{KR}}+3\left|\Delta\mu_{\mathrm{H}}^{\mathrm{e}}\right|_1\right)-1\right]. \quad (32)$$

When one compares φ_1=−6.7 and φ_2=−5.4, one should remember that the theoretical value of the electric potential is determined by the formula 9, which gives, in the limit of the little effect of the passive transport of potassium, the universal quantity $\varphi_{\mathrm{m}}=\Delta\mu_{\mathrm{H}}^{\mathrm{e}}$ weakly dependent on the proton flow through the ATP synthetase.

Analyzing results of the short circuit, positive ions are not pumped from mitochondria at the predominant passive transport of protons in terms of our model and, therefore, the distribution of ions on both sides of the membrane corresponds to the Boltzmann function. In this case, the neutrality condition in mitochondria gives the equation

$$n_{\mathrm{A}}^{\mathrm{i}}+n_{\mathrm{Cl}}^{\mathrm{o}}\exp(\varphi)=\left(n_{\mathrm{Na}}^{\mathrm{o}}+n_{\mathrm{K}}^{\mathrm{o}}\right)\exp(-\varphi), \quad (33)$$

which leads to the known Donnan potential. Substituting the concentration values, the formula 25 readily yields φ=−0.8, which obviously gives ΔpH=0.8 ($\Delta\mu_{\mathrm{H}}^{\mathrm{e}}=0$). Thus, the ΔpH value agrees well with experimental ΔpH=1.0.

The most considerable disagreement between the theory and the experiment consists in $\Delta\mu_{\mathrm{H}}^{\mathrm{e}}=-2.16$. In accordance with the theoretical model, K^+ is the only potential-forming positive ion. In the case of the short circuit, the difference of the chemical potentials of protons vanishes, while it is the only factor responsible for pumping potassium from mitochondria and generation of the potential (neglecting the Donnan potential). The experimental observation of φ=−3.16 suggests that mitochondria have one more unaccounted active mechanism of potassium transport.

2 Discussion

The proposed model of ion transport through the internal membrane in mitochondria allowed calculating concentrations of all ions (H^+, Ca^{+2}, Na^+) in the mitochondrion matrix and the membrane potential using known concentrations of ions in cells. The theoretical values are in satisfactory agreement with the available experimental data on the concentrations and the potentials, including different operating regimes of the ATP synthetase (the main regime, the short circuit, and blocking of the ATP synthetase). The concentrations of ATP, ADP, and P in mitochondria were calculated for the first time. The ATP synthetase reversibility, which is known from experiments, was confirmed and explained in terms of the theoretical model. The results obtained from the theoretical model suggest that normal functioning of mitochondria (ATP synthesis) is impossible in the absence of the active transport of magnesium ions. In accordance with the model, the ATP synthetase is operable only if the stoichiometric coefficient equals three (a single event of ATP synthesis involves three protons passing to the inside of a mitochondrion).

The measured difference of the electrochemical potentials of protons during blocking of the ATP synthetase was used to determine the difference of the chemical potentials in the Krebs cycle, $\Delta\mu_{KR}=27.6$ (neglecting the loss of protons for the operation of the exchangers and the passive transport). The calculations are in satisfactory agreement with the relevant experimental data only if the K^+–H^+ exchanger operates under saturation with K^+ ions. It would be interesting to prove this experimentally. The further elaboration of the model will include a more profound study of the regulating action of Ca^{2+} and the determination of the relative participation of different mechanisms of Ca^{2+}, K^+, and Na^+ transport.

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